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NSF-Independent Fusion Mechanisms

Minireview

Katherine L. Wilson
Department of Cell Biology and Anatomy
The Johns Hopkins University School of Medicine
Baltimore, Maryland 21205

The SNARE hypothesis provides an appealing framework for understanding the mechanism of vesicle targeting and fusion in the secretory and endocytic pathways (Rothman, 1994). In this model, vesicles bind specifically to their target membranes via complementary sets of membrane-specific SNAREs (soluble N-ethylmaleimide-sensitive factor [NSF] attachment protein [SNAP] receptors). Targeting specificity is also determined by the Rabs, a family of small soluble GTPases essential for vesicle transport (Novick and Brennwald, 1993; Simons and Zerial, 1993; Fischer von Mollard et al., 1994), which may regulate SNARE–SNARE interactions (Lian et al., 1994; Søgaard et al., 1994). Sec1-related proteins interact with both Rabs and t-SNAREs, and might also control specificity (Garcia et al., 1994; Pevsner et al., 1994; Søgaard et al., 1994). After specific vesicle docking is achieved, the SNARE complex recruits the soluble proteins α-SNAP, γ-SNAP, and NSF. The final product of this reaction, the SNARE–SNAP–NSF complex, is proposed to be a fusion machine.

NSF and the SNAPs play conserved and essential roles in membrane traffic. The ATPase activity of the trimeric NSF molecule is tightly coupled to membrane fusion in vitro (Whiteheart et al., 1994; Rothman, 1994). ATP hydrolysis by NSF is required to dissociate detergent-solubilized SNARE–SNAP–NSF complexes in vitro (Söllner et al., 1993). Although this is consistent with the idea that NSF (or the complex) triggers fusion directly, the identity of the fusogen(s) is not clear, and the fusion mechanism remains a mystery.

The SNARE hypothesis is proposed to cover all targeted fusion events during secretion and endocytosis. This minireview will highlight two exceptional fusion reactions in which the SNARE hypothesis may not apply. The first involves a type of fusion that was predictably distinct: homotypic endoplasmic reticulum (ER) membrane fusion (Latterich and Schekman, 1994). The second example is provocative because it involves a targeted fusion event: apical vesicle transport in Madin–Darby canine kidney (MDCK) cells (Ikonen et al., 1995 [this issue of *Cell*]). These results point to the existence of at least one NSF-independent fusion pathway in the cell.

Homotypic ER Membrane Fusion

SNARE and Rab proteins ensure that vesicles fuse vectorially during secretion and endocytosis. Thus, ER transport vesicles are targeted to *cis*–Golgi membranes. By contrast, ER tubules can also fuse with each other in living cells (Lee and Chen, 1988; Dabora and Sheetz, 1988), a process termed homotypic fusion. Mitochondria may also fuse homotypically (Bereiter-Hahn and Vöth, 1994). It has been proposed (Warren, 1993; Rothman and Warren, 1994) that a dynamic balance between homotypic fission and fusion might control organelle structure and might promote organelle distribution throughout the cell. Homotypic fission/fusion mechanisms are also thought to ensure that daughter cells inherit essential single-copy organelles during cell division.

Latterich and Schekman (1994) devised an assay for ER membrane fusion in vitro, using microsomes isolated from Saccharomyces cerevisiae. Their assay measures the transfer of glycosylated pro–α factor from the lumen of microsomes that lack the glucose-trimming enzyme (glucosidase I) to the lumen of microsomes containing wild-type enzyme, where sugar processing occurs. They made two key observations. First, ER homotypic fusion requires ATP but not cytosol. Cytosol does not stimulate the reaction, and membranes, pretreated with 3 M urea and 2 M potassium acetate to remove most associated cytosolic proteins, remained active for fusion. Second, membranes depleted of NSF (Sec18p) and α-SNAP (Sec17p) were inactive for ER-to-Golgi transport in vitro, as expected, but remained active for homotypic fusion. Sec18p and Sec17p were physically removed from *sec18* mutant membranes by sequential trypsin digestion, urea extraction, and salt extraction. Although this procedure might not remove other tightly bound cytosolic proteins, the evidence strongly suggests that ER homotypic fusion is independent of NSF and α-SNAP.

The mechanism of homotypic ER fusion is therefore different from that of ER-to-Golgi vesicular transport, which requires both NSF and α-SNAP. One burning question is the identity of the ER membrane-associated proteins required for homotypic fusion—are they SNAREs, or do they represent a new class of recognition/fusogenic proteins? Candidates for such membrane proteins were identified by Rose and colleagues, who developed a genetic screen for karyogamy mutants (Kurihara et al., 1994). Their data suggest that ER homotypic fusion mechanisms also mediate nuclear envelope fusion (termed karyogamy), which occurs after two yeast cells mate. Mutations in four genes were found to disrupt a late step in karyogamy that may correspond to the membrane recognition or fusion event: *kar2* (BiP), *kar7* (now known to be *SEC71* [M. Rose, personal communication]), and two other genes, *kar5* and *kar8*. Membranes isolated from *kar2*, *kar5*, and *kar8* mutants are defective for ER homotypic fusion in vitro (Kurihara et al., 1994) and are not rescued by the addition of wild-type cytosol. Information about the functions of Kar5p and Kar8p is eagerly awaited.

Organelle Assembly Mechanisms: Homotypic, NSF Dependent, or Unique?

The nuclear envelope, ER, and Golgi complex disassemble during mitosis in higher eukaryotes (Warren, 1993). The ER is structurally and functionally continuous with the outer nuclear membrane and lumen of the nuclear envelope. One might therefore expect that nuclear vesicle fusion, which occurs after mitosis to reassemble the nuclear envelope, would employ mechanisms similar to ER homotypic fusion. Based on the limited information avail-

able, however, the two fusion events may differ at least in terms of their regulation. As defined in yeast, ER homotypic fusion does not require cytosol (Latterich and Schekman, 1994). In contrast, postmitotic nuclear vesicle fusion in Xenopus egg extracts requires cytosol and also appears to involve a regulated Ca^{2+} release event mediated by inositol trisphosphate receptors, which are intracellular ligand-gated Ca^{2+} channels located on nuclear and ER vesicles (Sullivan et al., 1993; Sullivan and Wilson, 1994). Crudely based on its apparent requirements for cytosol and mobilized Ca^{2+}, nuclear vesicle fusion has more in common with regulated fusion events, such as synaptic vesicle fusion (Llinás et al., 1992), than with ER homotypic fusion. It is not yet known whether nuclear vesicle fusion requires NSF, SNAPs, or SNAREs. The relationship of nuclear vesicle fusion to either ER homotypic fusion or fusion within the secretory pathway is not clear. For example, the small GTPase, ADP-ribosylation factor (ARF), promotes vesicle formation at various stages in the secretory pathway (Boman and Kahn, 1995; Rothman, 1994); ARF can also bind nuclear vesicles and inhibit their fusion when irreversibly activated by GTPγS (Boman et al., 1992a). However, ARF plays no positive role in fusion, since fusion occurs normally in ARF-depleted reactions (Boman et al., 1992b). For functionally and structurally complex organelles such as the nuclear envelope and Golgi complex, it can be a challenge to understand why a given protein interacts with that organelle. For example, the Golgi complex has a core structure (Weidman et al., 1993) that is distinct from its peripheral trafficking regions and might therefore employ both homotypic and NSF-dependent fusion mechanisms during reassembly. By exploring the mechanisms of vesicle recognition and fusion during organelle assembly, we may discover new aspects of organelle structure and function.

A Surprise: Apical Transport in MDCK Cells Is NSF Independent

We will now consider the most startling case of NSF-independent fusion. Vesicle transport to the apical and basolateral cell surfaces of polarized cells can be studied using MDCK cells. The apical and basolateral membranes are functionally distinct and can be manipulated and assayed separately for the arrival of transported marker proteins. As reported by Ikonen et al. (1995), Simons and colleagues compared the molecular requirements of three transport steps in permeabilized MDCK cells: ER-to-Golgi transport, transport from the *trans*-Golgi network (TGN) to the basolateral membrane, and transport from the TGN to the apical surface. Both ER-to-Golgi transport and TGN-to-basolateral transport behaved as predicted by the SNARE hypothesis: transport was stimulated by recombinant α-SNAP, inhibited by antibodies to NSF, inhibited by Rab-GDI (which inactivates Rab proteins), and inhibited by neurotoxins that destroy the v-SNARE cellubrevin. In surprising contrast, apical transport was not affected by any of these treatments. This study is significant because of its internal controls—the analysis of ER-to-Golgi and TGN-to-basolateral transport in the same cells—and because apical transport was shown to be independent of four different components essential to the SNARE hypothesis: NSF, α-SNAP, Rab, and a v-SNARE. These results argue that apical transport (and perhaps other steps in forward or retrograde membrane traffic?) involves mechanisms that we do not yet understand.

Curiously, other work from Simons' group shows that apical transport is inhibited by antibodies to the unique N-terminus of annexin 13b (Fiedler et al., 1995). The annexins are a confusing family of Ca^{2+}-dependent phospholipid-binding proteins, some of which might function during exocytosis (Creutz, 1992). Annexins do not integrate across the lipid bilayer, but bind amphipathically to the cytoplasmic face of membranes. Some annexins have the properties of voltage-gated Ca^{2+}-selective ion channels in vitro (Demange et al., 1994). Although voltage gating is difficult to reconcile with intracellular fusion events, the putative in vitro ion channel activity might reflect a physiological role for annexins—namely, to destabilize phospholipid bilayers during membrane fusion. The potential role of annexins in apical transport raises an interesting question: do annexins play any role in NSF-dependent fusion reactions?

How Many Fusion Mechanisms Does It Take to Build a Cell?

The SNARE hypothesis has great predictive power. Nevertheless, the apparent lack of involvement of NSF and α-SNAP in ER homotypic fusion and, particularly, in apical transport reactions indicates that we do not yet have the whole picture. The SNARE hypothesis will provide a valuable tool for dissecting and comparing potentially novel fusion pathways. It will be essential to identify the proteins that mediate exceptional fusion reactions. These new proteins could be distant isoforms of NSF, SNAPs, and SNAREs that are insensitive to currently available antibodies and probes, or they could be entirely unrelated molecules. In either case, it will be interesting to figure out why the NSF-dependent pathway is not being used. Just as a single mutant can genetically reveal a new biological pathway, one or more of the exceptional assays may reveal a truly NSF-independent fusion reaction(s) with unique properties and enrich our understanding of the cell.

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